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Mutual Functional Destruction of HIV-1 Vpu and Host TASK-1 Channel

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Summary

Sequence analysis predicted significant structural homology between the HIV-1 accessory protein Vpu and the N-terminal region of TASK-1, a mammalian background K^+ channel. If the homology resulted from molecular piracy during HIV-1 evolution, these two proteins may have important functional interactions. Here we demonstrate that TASK and Vpu physically interact in cultured cells and in AIDS lymphoid tissues. The functional consequences were potentially destructive for both components: Vpu abolished TASK-1 current, while overexpressing TASK led to a marked impairment of Vpu's ability to enhance viral particle release. Further, the first 40 amino acids of TASK-1 (part of the homology to Vpu) were capable of enhancing HIV-1 particle release. This virus-host interaction may influence HIV-1/AIDS progression, as well as electrical signaling in infected host tissues.

Introduction

HIV-1 proteins constantly interact with host components in infected cells (Greene and Peterlin, 2002). How such interactions emerged during the course of virus evolution is not clear. One common theme in virology is that of "molecular piracy"—viral components may evolve from replication of related host genes, thus being capable of interacting with native proteins (Sinkovics et al., 1998). For molecular piracy, cellular immunomodulatory genes are popular targets; this is well illustrated in cytomegaloviruses (CMVs), which incorporate homologs to host MHC class I, chemokines, and G protein-coupled receptors (Vink et al., 1999). Here we report an unusual example of virus mimicry using an ancient K^+ channel as a template. Significant sequence homologies were found between the HIV-1 Vpu protein and the N-terminal 70 residues of the two-pore K^+ (K_{2P}) channel TASK-1.

TASK-1 is a widely expressed, acid-sensitive K^+ channel that exhibits background conductance (Duprat et al., 1997; Kim et al., 1998; Leonoudakis et al., 1998). Vpu plays multiple roles during HIV-1 infection: support of virus release (Klimkait et al., 1990; Strebel et al., 1989) and promotion of CD4 degradation (Willey et al., 1992). This small integral membrane protein of HIV-1 also influences cationic currents in planar lipid bilayers and in *Xenopus* oocytes (Coady et al., 1998; Gonzalez and Carrasco, 1998; Schubert et al., 1996). This electrophysiological property of Vpu requires conservation of the transmembrane (TM) sequence. Since Vpu is multimeric in native states (Maldarelli et al., 1993), it has been proposed to form ion channels (Schubert et al., 1996), but it is also possible that Vpu affects membrane conductance through modulation of endogenous ion channels (Lamb and Pinto, 1997), as do other small integral membrane proteins (Abbott and Goldstein, 1998; McDonald et al., 1997; Sesti et al., 2000; Shimbo et al., 1995). In this report, we provide the first evidence that HIV-1 Vpu alters the normal function of cellular channel subunits by direct interaction with those subunits.

Using a number of biochemical and biophysical approaches, we first demonstrated that TASK and Vpu interact in heterologous expression systems and in HIV-1-infected specimens. The functional consequences of such interaction are mutually destructive: Vpu abolishes the background conductance of TASK-1, while TASK expression also suppresses Vpu-mediated virus release. Since K^+ channel subunits have the propensity to oligomerize with homologous subunits to form a functional pore (e.g., hetero-dimerization of TASK-1 and TASK-3 [Czirjak and Enyedi, 2002]; hetero-tetramerization of "silent subunits" to Kv 2.1 subunits [Ottschytsch et al., 2002]), TASK, and Vpu may interact through the same mechanism as channel assembly. By intercalating into TASK complexes, Vpu inhibits the background current generated by TASK channels. Conversely, it is conceivable that, if HIV-1 infection takes place in cells with substantial TASK expression, the amount of Vpu trapped by TASK could become significant enough to affect viral particle release.

The first transmembrane segment is essential for assembly of voltage-dependent K^+ (Kv) channel subunits (Babila et al., 1994). A truncated Kv channel with only the first transmembrane segment (TM1) remains capable of homo- or hetero-multimerization. Coexpression of these TM1 fragments with wild-type Kv channel subunits exerts a negative *trans*-dominant effect on the currents encoded by wild-type channels. Since these TM1 fragments can spontaneously assemble with native homologous Kv subunits (likely during cotranslation [Deutsch, 2002]), their oligomerization results in nonfunctional channel complexes on the cell surface or retention in the ER (Folco et al., 1997). If we envision Vpu as a mimic of the TM1-containing TASK fragment, its inhibition of TASK function may be mechanistically similar to inhibition of Kv channels by their TM1 fragments.

We therefore tested whether the first TM region of TASK (termed "Ttm1") could functionally mimic Vpu. Not only did Ttm1 suppress TASK conductance, but it also enhanced HIV-1 release. Hence, the minimal structural requirement for Vpu-mediated virus release is equivalent

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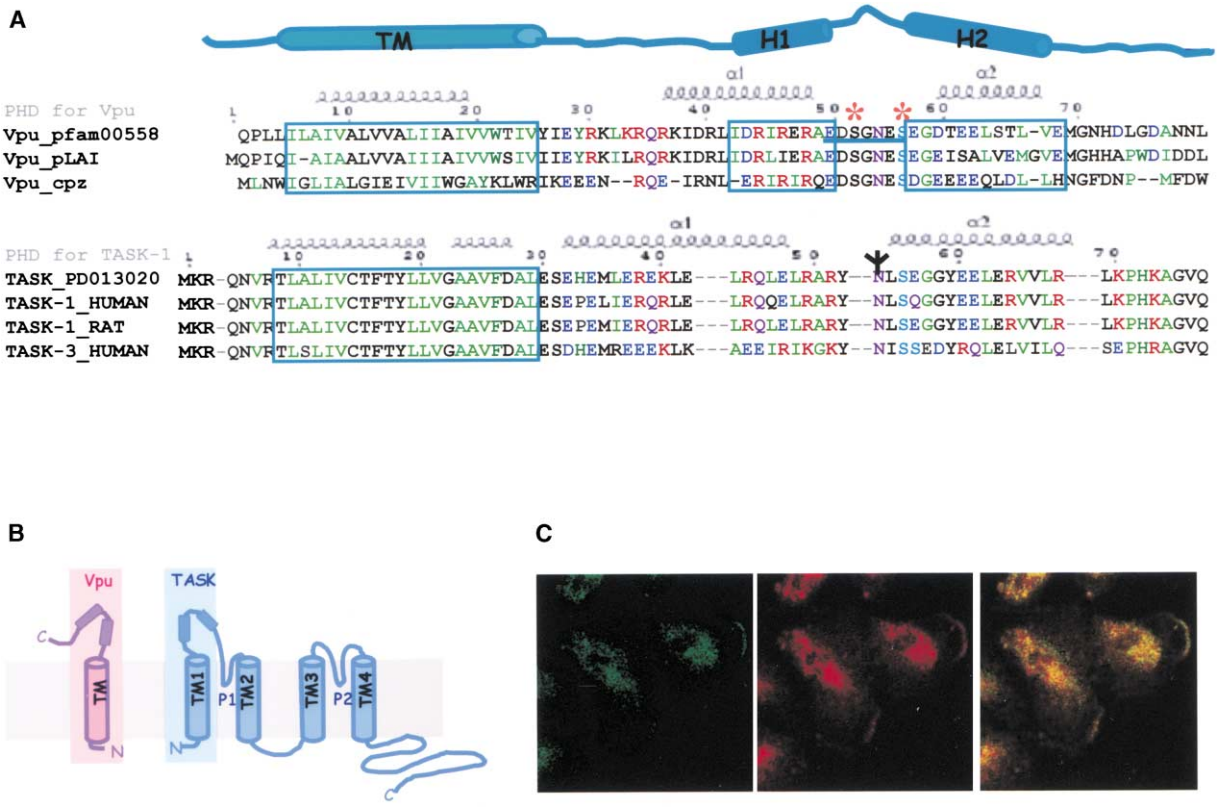

Figure 1. TASK-Vpu Homology Leads to Their Physical Association

(A and B) Protein sequence alignment between Vpu and the first quarter of TASK. PHD-predicted helices (including TMH) are indicated by gray symbols. The Vpu alignment includes the consensus sequence from NCBI Conserved Domain Database (CD: pfam00558), the sequence from pLAI plasmid, and another consensus from the rare SIVcpz strains compiled by the HIV Database. PD013020: the TASK-1 consensus sequence from ProDom database. In Vpu, the hinge region linking the 2 non-TM helices (blue underline and boxes) contains two phosphoserines (red asterisks) that are important for CD4 degradation. A potential N-linked glycosylation site is conserved in TASK (branch). Aligned amino acids are color coded by MView 1.41 (positive charge, bright red; negative charge, bright blue; polar, purple; small alcohol, dull blue; hydrophobic, bright green; large hydrophobic, dull green).

(C) Colocalization of GT (green) and CD4U (red): shown both individual scans and the overlay.

to a segment of the host K^+ channel that is essential for channel assembly. Consequently, Vpu expression in HIV-1-infected cells disrupts the cellular pathway for K^+ channel biogenesis. The resultant changes in the transmembrane electric field induced by the TASK-Vpu interaction are expected to alter conformations of membrane proteins, membrane fluidity and structure (Lakos et al., 1990; Lelkes, 1979), as well as intracellular ion homeostasis, undermining efficient virus packaging and release.

Results

Proposal for Molecular Piracy of Cellular TASK-1 Channels

Significant sequence homologies were identified between HIV-1 Vpu and the first quarter of mammalian TASK-1 with overall identities of 24% (16/68) and sequence conservation of 56% (38/68) (Figures 1A and 1B). This degree of sequence similarity is comparable to that among different subfamilies of K_{2P} channels. As the evolution of K_{2P} channels probably spanned a much longer time frame than that of Vpu, it is anticipated that the rates of their sequence divergence were different, and that a virus-specific evolutionary mechanism such as molecular piracy was involved.

In addition to pairwise comparison, the neural-net-based PHD program also predicts a similar folding pattern in the aligned regions (transmembrane helix-helix-loop-helix) for both proteins. While the secondary structure of TASK-1 is unknown, the PHD prediction for Vpu matches its post-TM structure resolved by NMR (Wray et al., 1995) (Figure 1A).

The high degree of Vpu-TASK homology, and the fact that both proteins have the ability to oligomerize, suggested that these two proteins might interact to form hetero-oligomers. We used confocal imaging as a means to verify TASK-Vpu association. A GFP-TASK fusion (GT) and the CD4U chimera (CD4 ectodomain tagged to Vpu) were used (Bour et al., 2001). Both CD4U and GT are functionally equivalent to the untagged Vpu and TASK-1, respectively: CD4U maintains full Vpu functionality—enhancement of virus release and modulation of CD4 degradation (Bour et al., 2001; Paul et al., 1998), while GT exhibits K^+ conductance equivalent to that of TASK-1 (data not shown). Coexpression of CD4U and

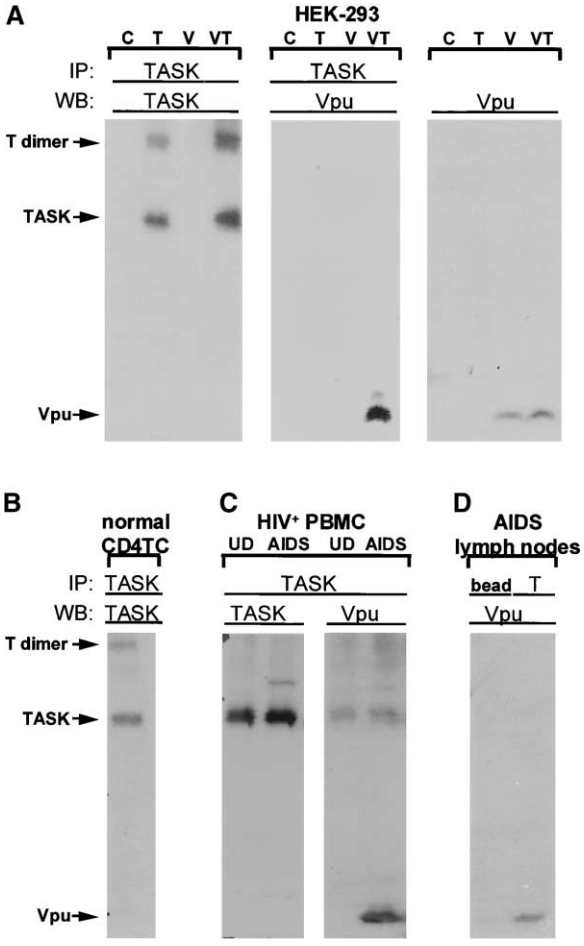

Figure 2. Coimmunoprecipitation of TASK-1 and Vpu

(A) Vpu was identified as part of the TASK complexes in HEK-293 cells expressing both TASK and Vpu (VT). Negative controls include expression of the control GFP alone (C), TASK-1 alone (T), and Vpu alone (V).

(B) TASK expression (55 kDa) in PHA-activated normal primary $CD4^+$ T lymphocytes.

(C) TASK-Vpu interaction detected in an AIDS peripheral blood sample (AIDS), and not in the peripheral blood from a treated HIV-1-positive patient with an undetectable viral load (UD).

(D) T-V interaction found in AIDS autopsied lymph node tissues. "Bead" (negative control): not chemically coupled to anti-TASK.

GT in A549 cells revealed that these two proteins were colocalized intracellularly and on the plasma membrane (Figure 1C). Despite the inability to achieve molecular resolution by confocal imaging, the spatial correlation of TASK and Vpu provides a basis for interpretation of their functional interactions as described below.

TASK-Vpu Protein-Protein Interaction

We characterized the TASK-Vpu protein-protein interaction by coimmunoprecipitation. Anti-TASK-1 antibody, which recognizes a C-terminal peptide far from the region homologous to Vpu, was used to immunoprecipitate TASK-1 complexes under conditions of mild detergent dissociation. For HEK-293 cells expressing TASK (T, TASK-alone; VT, TASK + Vpu), Western blot revealed monomeric and dimeric TASK bands in their immunoprecipitates (Figure 2A, left panel). Vpu expression was not affected by cotransfection of TASK (Figure 2A, right panel). Indeed, Vpu was only identified in the TASK precipitates from the VT sample (Figure 2A, middle panel), indicating that these two proteins form stable oligomers through heterologous expression. Interestingly, the U2-3 anti-Vpu antiserum was unable to coimmunoprecipitate TASK, suggesting that, under our experimental conditions, the U2-3 epitope in the Vpu cytoplasmic tail was masked. Therefore, both the transmembrane segment and cytoplasmic tail of Vpu may be involved in TASK interaction.

To determine whether this interaction is meaningful in the context of HIV-1 infection, we first characterized the expression level of TASK-1 in phytohemagglutinin (PHA)-activated primary $CD4^+$ T lymphocytes (Figure 2B). Further tests on AIDS lymphoid tissues (peripheral blood and autopsied lymph nodes) revealed the existence of the TASK-Vpu interaction in vivo (Figures 2C and 2D). The interaction was examined in blood samples from an AIDS patient and from a treated HIV^+ patient with undetectable viral load (UD). The expression levels of monomeric and dimeric TASK (55 kDa, 110 kDa) in both samples were comparable. Only in AIDS blood was Vpu identified in the TASK oligomers. Western blots against Vpu also frequently identified TASK-sized bands on native samples; this crossreactivity may be explained by the overall homology of Vpu to the first quarter of TASK-1, and further implies a structural resemblance between the two proteins in vivo.

Suppression of TASK-1 Current by Vpu Coexpression

To identify the functional consequences of TASK-Vpu oligomerization, we first dissected the background conductance of TASK in the presence or absence of Vpu in heterologous expression systems (HEK-293, A549). Since TASK-1 neither displays detectable kinetics nor has a specific channel blocker, two methods of measurement were employed to differentiate its current from endogenous currents in a cell. The first method measured TASK conductance by differential blockade using two K^+ channel blockers, Ba^{2+} and TEA^+. Because TASK-1 can be completely blocked by Ba^{2+}, but not TEA^+ (Duprat et al., 1997; Kim et al., 1999), we employed stepwise blockade in a K^+-free bath condition at 0 mV to suppress any endogenous currents (Backx and Marbán, 1993). A 1:1 molar ratio of TASK and Vpu DNA was applied in transfection. HEK-293 cells expressing TASK-1 alone exhibited a sizable Ba^{2+}-sensitive, TEA^+-insensitive outward current (Figure 3A, inset: T). However, coexpression of TASK and Vpu completely suppressed this characteristic TASK-1 current component (Figure 3A, inset: VT). Combined with the coimmunoprecipitation results, the findings indicate that the TASK-1 channel becomes functionally impaired following oligomerization with Vpu.

The second method measured TASK-1 by activating its pH-sensitive current. TASK-1 is sensitive to extracellular pH changes in the physiological range; the current can augment 50% from pH 7.5 to pH 8.5, at which point it saturates (Duprat et al., 1997; Leonoudakis et al., 1998). We therefore examined whether the pH-sensitive component of TASK (subtracting the value at pH 7.5 from that at pH 8.5) was affected by Vpu. To differentiate

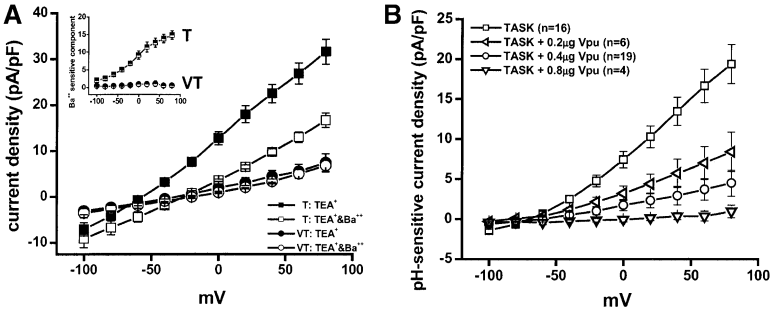

Figure 3. Vpu Coexpression Inhibits TASK-1-Specific Current

(A) Coexpression of TASK and Vpu abolished the Ba^{2+}-sensitive, TEA^{+}-insensitive component of TASK-1 current. HEK-293 cells transfected with TASK-1 alone (T) or with both TASK-1 and Vpu (VT) were voltage-clamped under whole cell configuration. The cells were perfused from K^{+}-containing bath solution to the same solution eliminating K^{+}, then with an addition of 10 mM TEA^{+} (solid symbols). Continued perfusion with additional 2 mM Ba^{2+} (hollow symbols) reveals the Ba^{2+}-sensitive and TEA^{+}-insensitive K^{+} conductance, specific of TASK-1 (square; mean ± SEM). This conductance component was absent with Vpu coexpression (circle; mean ± SEM). Inlet: Subtraction for the Ba^{2+}-sensitive, TEA^{+}-insensitive conductance.

(B) TASK-Vpu coexpression also suppressed the pH-sensitive component of TASK current, activated by pH increase from 7.5 to 8.5. Shown dose dependence of Vpu-imposed inhibition of TASK-1 current.

between specific channel inhibition by Vpu and cytotoxic effects, a dose-response experiment was performed in which TASK current was measured in the presence of increasing amounts of transfected Vpu. The results correlate well with the previous measurements by differential blockade: Vpu coexpression significantly suppressed the TASK-specific current (Figure 3B). Although further increase of Vpu DNA completely inhibited the pH-sensitive TASK current, it also made the cells difficult to patch, presumably due to cytotoxic effects of Vpu overexpression (Figure 3B).

To exclude the possibility that the observed suppression of TASK-1 currents by Vpu results from Vpu-induced toxicity and not from hetero-oligomerization, in the second patch-clamp experiment (Figure 3B), we identified apoptotic cells with two markers: Alexa Fluor-conjugated anti-annexin V and propidium iodide. Despite the fact that immunostaining prior to electrophysiological recording revealed some Vpu-expressing, apoptotic cells, the majority of such cells did not attach to coverslips well and were quickly washed away during perfusion of the recording chamber. We also compared the surface areas of all patched cells for signs of cell shrinkage or apoptotic volume decrease, a prerequisite for the onset of apoptosis. Vpu coexpression did not affect the surface area of HEK (data not shown). Additionally, suppression of TASK conductance by Vpu appeared consistent throughout the whole time course of transient expression (data not shown). The results suggest that coassembly and functional interference begin before TASK is functionally mature, and do not result from Vpu-induced apoptotic effects.

Inhibition of Vpu-Mediated Virus Release by TASK

The TASK-Vpu protein-protein interaction may also affect Vpu functionality. Of the two functions of Vpu, promotion of CD4 degradation and enhancement of virus release, we chose to focus on the latter for two reasons. First, Schubert et al. (1996) have correlated the putative channel-like activity of Vpu to its regulation of HIV-1 release. Second, our confocal imaging and whole-cell electrophysiological studies both point to a functional interaction of TASK-1 and Vpu at the level of the plasma membrane, the reported site of Vpu-mediated particle release enhancement (Bour et al., 1999). Therefore, if Vpu inactivates TASK by disrupting oligomeric structures necessary for the formation of a functional channel, the reciprocal effect might be observed in circumstances where TASK is in excess over Vpu.

To determine the effect of TASK-1 on virus release, HeLa cells were cotransfected with wild-type and Vpu-defective HIV-1 proviral constructs (pNL4-3 and pNL4-3/Udel, respectively). Viral contents in culture media were determined at 25 hr posttransfection. The particle release efficiency of NL4-3 alone was set to 100%. As previously reported (Strebel et al., 1988), inactivation of the *vpu* gene in the NL4-3 proviral clone of HIV-1 dramatically reduced the efficiency of particle release (Figure 4A, pNL4-3/Udel). TASK-1 coexpression with wild-type pNL4-3 reduced virus release by 2- to 3-fold (Figure 4A, pNL4-3 + TASK). The suppressive effects of TASK-1 on viral particle release did not result from a general overexpression of K^{+} channels, as demonstrated in the negative control using another K^{+} channel of comparable current density, HERG (Figure 4A, pNL4-3 + HERG). 2-fold suppression of HIV-1 release was observed when applying 0.7 μg of TASK DNA (equivalent to the usage in electrophysiological studies [Figure 3]). A dose-response study of TASK-mediated inhibition of particle release showed up to 3-fold inhibition of particle release, with a minimal effect on total viral protein production (Figure 4B). On the other hand, TASK-1 coexpression with pNL4-3/Udel lacking Vpu did not affect virus release (Figure 4A, pNL4-3/Udel + TASK). Therefore, the suppressive effect of TASK-1 on HIV-1 is Vpu specific. These data further reinforce the notion that Vpu and TASK engage in hetero-oligomer formation, disrupting their individual multimeric structures. While it is likely that, in actively HIV-1-infected cells, the Vpu/TASK ratio is in favor of Vpu and that most of the inhibitory effect would be directed against TASK, overexpressing TASK has allowed us to further characterize the mechanism of TASK-Vpu interaction.

Vpu as a Mimic of the First Transmembrane Segment of TASK

There is much resemblance between the TASK-Vpu interaction and the effect of a truncated Kv channel polypeptide on native Kv channels (Babila et al., 1994). An N-terminal TM1-containing fragment of Kv1.1 was

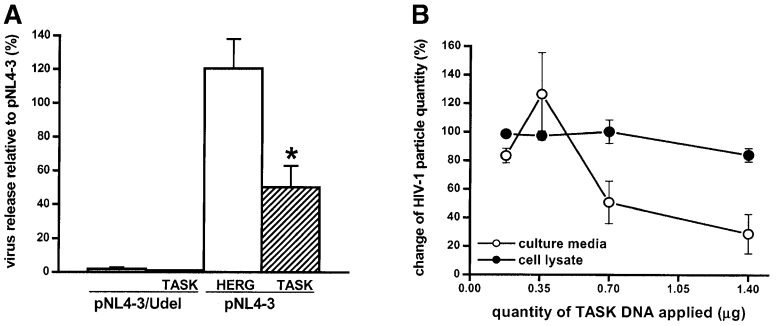

Figure 4. TASK-Vpu Interactions Interfere with HIV-1 Release

(A) TASK-1 coexpression with pNL4-3 suppressed virus release, as compared with the control HERG channel. Asterisk designating $p < 0.05$ in a Student's *t*-Test is the significance of a comparison of virus release affected by TASK-1 (pooled data) to that by HERG, from seven independent experiments. Deletion of Vpu (pNL4-3*Udel) resulted in inhibition of virus release (mean ± SEM, n = 3). (B) Dose dependence of TASK-1 inhibition on Vpu-mediated virus release.

shown to spontaneously oligomerize with native Kv1 subunits in vitro (Folco et al., 1997) and in transgenic mice (London et al., 1998). Such coassembly resulted in inhibition of endogenous Kv conductance, presumably due to the formation of misfolded and nonfunctional hetero-oligomers.

If the homology between HIV-1 Vpu and TASK is indeed evolutionarily meaningful, the inhibitory mechanism of Vpu on TASK may resemble that whereby a truncated Kv polypeptide suppresses native voltage-gated K^+ channels. First, to establish that TASK can also be inhibited by its N-terminal TM-containing fragment, we constructed a TASK-1 truncation that comprises the NH_2 terminus and the first TM segment ("Ttm1"). Coexpression of Ttm1 with TASK suppressed TASK current, in analogy with previous observations for voltage-gated K^+ channels (Babila et al., 1994). More importantly, the suppression of TASK current by Ttm1 closely resembled that by HIV-1 Vpu (Figures 5 and 3B).

The dose-response analysis, however, reveals that the mechanism of TASK inhibition by Ttm1 is not identical to that by Vpu. Ttm1 overexpression did not completely eliminate the TASK-1-specific current. The maximal Ttm1-imposed inhibition was reached with 0.2 μg of Ttm1 DNA in cotransfection (DNA molar ratio of TASK: Ttm1 ≈ 2: 1). Further increase of Ttm1 doses did not enhance suppression of TASK, and there was always some residual TASK-1-specific current (Figure 5). On the other hand, Vpu-imposed inhibition of TASK appears to be directly proportional to the quantity of Vpu DNA applied in transfection. Unlike Ttm1-imposed inhibition, increasing Vpu quantities in transfection could completely suppress TASK current (TASK: Vpu ≈ 1: 2) (Figure 3B).

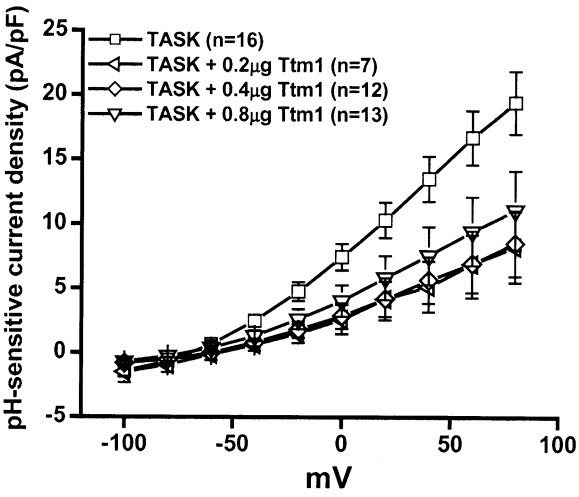

Figure 5. Ttm1 Coexpression Suppressed the pH-Sensitive Current of TASK-1

Dose dependence of Ttm1-imposed inhibition of TASK-1 current. The molar ratio of TASK (0.4 μg): Ttm1 (0.4 μg) is roughly 1:1.

TASK Degradation during HIV-1 Infection

Although the mechanism previously invoked to account for TASK inhibition by Vpu could only involve disruption of the TASK complexes, Vpu is also known to function as a connector between target proteins and the ubiquitin-proteasome degradation pathway. It is therefore conceivable that Vpu-TASK interactions lead to the recruitment of the SCF^{TrCP} E3 ligase and degradation of TASK, as observed in the case of CD4 (Margottin et al., 1998). To address this possibility, we monitored TASK-1 expression in primary lymphocytes during HIV-1 infection. $CD4^+$ T lymphocytes on the third day of PHA activation were infected with HIV-1 by spin inoculation (Pierson et al., 2002). On day 2 or 3 postinfection, endogenous TASK-1 was extracted and compared among the three groups: infected cells, plus uninfected (control) cells at t = 0 and at t = 48–72 hr postinfection. TASK expression was significantly reduced in HIV-1 infected cells. The two groups of uninfected cells exhibited similar TASK levels, indicating that PHA activation after day 3 did not affect endogenous TASK expression (Figure 6A). Despite the decreasing expression of TASK in the infected sample, Vpu was still identified in the TASK complexes from immunoprecipitation.

We have also identified βTrCP, a component of the ubiquitin-proteasome degradation pathway that binds to Vpu (Margottin et al., 1998), in the TASK precipitates from AIDS samples (Figure 6B). Taken together, the physical associations among TASK, Vpu, and βTrCP during HIV-1 infection may lead to acceleration of TASK degradation in primary cells. The data also suggest that Vpu-imposed inhibition of TASK conductance (Figure 3) occurs at least partly through acceleration of TASK degradation.

Ttm1 as Functionally Equivalent to Vpu in HIV-1 Release

Our findings that Vpu inhibits TASK activity might conceivably be used to further define the mechanism by which Vpu enhances viral particle release and to gain a

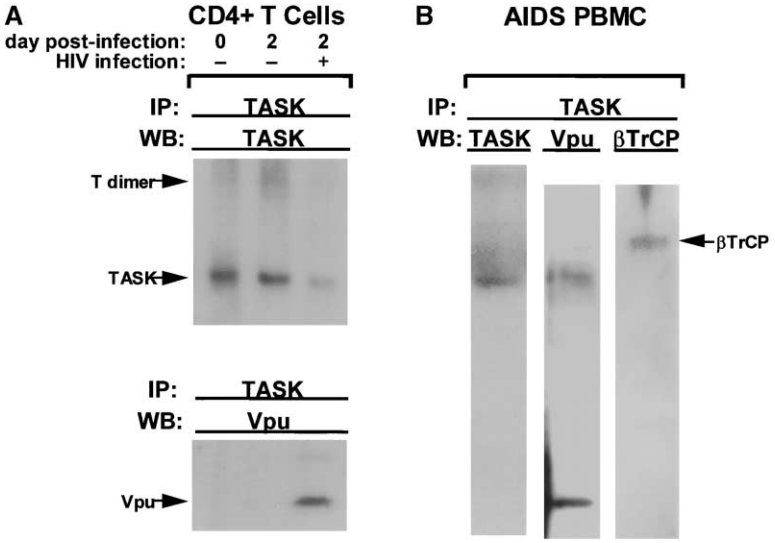

Figure 6. TASK-1 Degradation during HIV-1 Infection

(A) Endogenous TASK expression is diminished in HIV-1 infection. PHA-activated primary CD4$^+$ T cells were infected with HIV-1 in vitro. The protein level of TASK-1 was assessed on day 0 and day 2 postinfection. Immunoprecipitation followed by Western blot revealed decreasing TASK expressions in infected cells, compared to the controls. TASK-Vpu association was also detected in in vitro infected primary cells.

(B) Coimmunoprecipitation of TASK-1, Vpu, and βTrCP in an AIDS peripheral blood sample.

better understanding of the evolutionary origin of this HIV-1-specific protein. If Vpu was indeed pirated from TASK, its major function—support of particle release—may have a structural root in host TASK. As Vpu-mediated viral release requires conservation of the Vpu TM region, the structural root of this function could be the first transmembrane region of TASK. Therefore, we extended our investigation of Ttm1 to viral particle release.

To determine whether Ttm1 could mimic the effects of Vpu on viral particle release, Ttm1 was coexpressed with the Vpu-defective pNL4-3/Udel. Virus release efficiency of NL4-3/Udel was set as the baseline (Figure 7: fold = 1). Transfection of as little as 0.18 μg Ttm1 DNA significantly enhanced HIV-1 release. This enhancer effect of Ttm1 was comparable to that of Vpu applied *in trans* to pNL4-3/Udel (Figure 7). Although Ttm1 seems to be more effective than Vpu in suppressing viral release at 40 hr posttransfection, differences persist throughout the entire time course, with an eventual reversal of the apparent efficacy; this suggests that the kinetics of protein synthesis and lifetime may differ between Vpu and Ttm1. We also observed a gradual diminution of the Ttm1 enhancer effect with increasing Ttm1 doses (data not shown). This is possibly contributed by a cytotoxic effect of Ttm1 overexpression, as overexpression of Vpu also imposes considerable cytotoxicity (Akari et al., 2001).

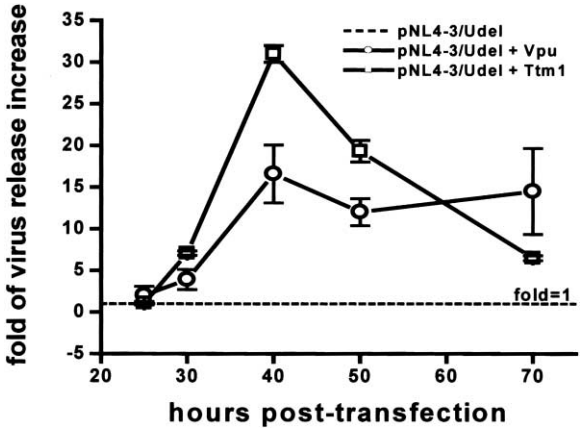

Figure 7. Ttm1 Is Functionally Equivalent to Vpu in Assisting HIV-1 Release

Ttm1 coexpression with pNL4-3/Udel supported viral particle release in HeLa cells, as did Vpu. Folds of increase of virus release were determined by comparison to the coexpression of pNL4-3/Udel and pCGI (fold = 1). Data summarized from two to five independent experiments.

Discussion

This work began with the hypothesis that the mammalian K$^+$ channel TASK-1 could oligomerize with HIV-1 Vpu given their structural homology (Figure 1). We indeed found the TASK-Vpu interaction in vitro, and in lymphoid tissues from AIDS donors (Figures 2 and 6). Functional studies point to bidirectional destruction as a consequence of oligomerization: HIV-1 Vpu could inhibit host TASK current (Figure 3), while TASK expression could also restrain Vpu-mediated virus release (Figure 4). Due to the ubiquitous expression of TASK in tissues (heart, brain/neurons, lung, kidney/glomerulosa, placenta, peripheral blood, and CD4$^+$ T lymphocytes), the consequences of the TASK-Vpu interactions are likely multifold and clinically relevant.

Their disproportionate protein sizes suggest that Vpu likely acts as a "viral auxiliary subunit" to TASK. Many K$^+$ channel complexes have been shown to incorporate small integral membrane proteins that contain a single transmembrane span. Coassembly of the main channel α-subunits with such auxiliary subunits often modify the original channel properties (Abbott and Goldstein, 1998; McDonald et al., 1997; Sesti et al., 2000; Shimbo et al., 1995). This is well illustrated by the interaction between the cardiac K$^+$ channel HERG and a single TM protein, MiRP1 (Abbott et al., 1999). Besides functional modulation, auxiliary subunits may also influence the biogenesis/trafficking of channel α-subunits.

On the other hand, the global homology of Vpu to the first quarter of TASK implies that the basis of their coassembly is similar to the basis of oligomerization of

homologous channel subunits, provided that the TM1-containing fragment is essential for K^+ channel assembly (Babila et al., 1994). We have indeed observed functional mimicry between Vpu and the first TM segment of TASK-1 (Ttm1): both are capable of inhibiting TASK-1 current and promoting viral particle release (Figures 5 and 7). These similarities support our initial conjecture that Vpu was pirated from host TASK during the evolution of HIV-1. Upon viral infection, the cellular machinery for TASK or K^+ channel ontogeny is co-opted and redirected by Vpu. We indeed observed substantial TASK degradation in primary $CD4^+$ T cells infected with HIV-1 in vitro (Figure 6).

The mechanisms that Vpu can employ to impair TASK currents were deduced by comparing the dose-response relationships of Ttm1- and Vpu-imposed inhibitions (Figures 3B and 5). While overexpression of Vpu could completely eliminate TASK current, application of Ttm1 at any dose could not. The differences are likely attributable to the non-TM sequence of Vpu that Ttm1 lacks. Ttm1 overexpression at most results in intracellular accumulation of full-length channel subunits and reduction of functional TASK. On the other hand, the Vpu cytoplasmic tail interacts with βTrCP in the ubiquitin-proteasome degradation pathway, and it is this interaction that promotes the degradation of CD4 in infected T lymphocytes. Endogenous TASK-1 probably is targeted for degradation in the same way during HIV-1 infection (Figure 6). Thus, Vpu is capable of (1) limiting TASK function through transmembrane interactions, and (2) accelerating TASK degradation through its cytoplasmic association with the ubiquitin-degradation pathway. The latter is analogous to HIV-1 Vif-induced proteasome degradation of the host antiviral enzyme APOBEC3G (Marin et al., 2003; Sheehy et al., 2003; Stopak et al., 2003). Future studies using the proteasome inhibitors, *trans*-dominant TrCPΔF, and Vpu26 will determine whether SCF-TrCP assists Vpu in degradation of TASK (Bour et al., 2001; Margottin et al., 1998).

It seems paradoxical that Ttm1 appears more effective than Vpu in suppressing viral release (at 40 hr post-transfection, Figure 7), but Ttm1 inhibits TASK channel activity to the same extent as does Vpu (Figures 5 and 3B). While Ttm1 is specifically designed to interact with TASK, Vpu has a number of cellular targets other than TASK; thus, TASK may be competing with other proteins for interaction with Vpu. Alternatively, the discrepancy may simply reflect different synthesis and/or turnover rates between Vpu and Ttm1.

TASK-Vpu oligomerization potentially destroys the individual normal functions of the two proteins. The functional implications are multiple, and are determined by two factors: (1) the divergency of the TM sequences of Vpu; and (2) their relative steady state levels. First, as suggested in the Ttm1 experiments (Figures 5 and 7), the transmembrane association between TASK and Vpu is the key to the bidirectional functional interference. We indeed found that some degree of randomization of the Vpu TM sequence could weaken its physical and functional interaction with TASK (see Supplemental Figure S1 at http://www.molecule.org/cgi/content/full/14/2/259/DC1), compounding the pathological complexity that may result from TASK-Vpu interactions.

The second factor determining TASK-Vpu association is their relative steady state protein expressions. Previous studies of HIV-mediated inhibition of cellular protein function have shown a tight dependence on the ratio between the viral effector and the cellular target. For example, the gp160 viral envelope protein precursor could form intracellular complexes with the CD4 receptor, leading to trapping of CD4 in the endoplasmic reticulum and complete downregulation from the cell surface (Bour et al., 1991). However, this mechanism relies on the fact that levels of gp160 in HIV-infected cells were several-fold higher than that of CD4. Artificially reversing this ratio restored CD4 at the cell surface and led to quantitative trapping of gp160 in the ER instead (Buonocore and Rose, 1990). As TASK expression varies in different specialized cells, and Vpu expression also varies depending on infection conditions, the outcomes of their interaction would not be expected to be the same in various HIV-1 accessible cell types. At a low level of TASK expression, such as in primary T lymphocytes, TASK-1 may be rapidly degraded when wrestling with Vpu during infection. In other HIV-1 reservoirs, such as neurons (Bagasra et al., 1996) and kidney (Winston et al., 2001), the high expression of TASK-1 (Talley et al., 2001) may pose an inhibitory effect on the spread of viruses, at the expense of having TASK channels degraded at an abnormal rate. The counteracting effects of TASK-1 on Vpu seemingly negate any selective advantage attributable to molecular piracy. In any case, this molecular pas de deux may ultimately serve to stabilize the virus-host relationship, facilitating the cellular and anatomical reservoirs of HIV-1 in infected patients (Bagasra et al., 1996; Bour and Strebel, 2003; Greene and Peterlin, 2002; Winston et al., 2001).

Experimental Procedures

Plasmids

The coding sequence of rat TASK1 (AF031384) was subcloned into the expression vector pCGI (pGFPIRS [Johns et al., 1997]) to generate pCGI-TASK1. The GFP-TASK1 fusion construct (pGT) was made by subcloning rat TASK1 into pEGFP-C2 (CLONTECH). The coding sequence of Vpu from the HIV-1 PLAI plasmid was subcloned into two similar bicistronic vectors: pCCI (CFP instead of GFP) and pAdC8I, which has CD8 as the reporter gene (Hoppe et al., 1999). Ttm1, a naturally occurring TASK-1 mutant comprising the first 40 residues with point mutation E37A, was subcloned into pCGI (pCGI-Ttm1).

Antibodies

The polyclonal rat anti-TASK1 antibody was custom produced against a synthetic C-terminal peptide of human TASK1 (EDEKRDAEHRALLTRNGQ) by BIOSYNTHESIS, INC., and further purified through a peptide-specific column (AminoLink Plus Immobilization kit, Pierce). The rabbit anti-Vpu antibody (U2-3) was made as described previously (Maldarelli et al., 1993).

Cells, Specimens, and Infection

HEK-293 and A549 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 5 μg/mL plasmocin (ant-mpp, InvivoGen, San Diego, CA). HeLa cells were maintained in Eagle's minimum essential medium (EMEM) supplementing with 1× nonessential amino acids, same FBS and antibiotics. The autopsied lymph nodes retrieved by National Disease Research Interchange were from an AIDS donor who refused any antiretroviral treatments. PBMC obtained from HIV-negative donors were maintained in STCM (RPMI, 10% fetal calf serum, interleukin-2 [100 U/ml], T cell growth factor), followed by 3 days of phytohemagglutinin activation and depletion of $CD8^+$ cells. Primary $CD4^+$

T lymphocytes were HIV-1 infected on the third day of PHA activation by 2 hr of spin inoculation, as previously described (Pierson et al., 2002), followed by 2 to 3 days of incubation.

Immunohistochemistry and Confocal Microscopy

A549 cells were cotransfected with pCD4U and pGT using LipofectAMINE Plus (Invitrogen). Within 36–48 hr, CD4U was immunolabeled with T4-4 antibody followed by Alexa Fluor 568-conjugated anti-rabbit antibody (Molecular Probes); the green Fluorescence of GT was enhanced by labeling with Alexa Fluor 488-conjugated anti-GFP antibody. Confocal images were taken on a Zeiss LSM 410 confocal microscope.

Coimmunoprecipitation and Western Blot

Heterologous expressions in HEK-293 cells were introduced by transient transfection. Within 36–48 hr posttransduction, these cells were lysed in digitonin lysis buffer (0.8–1% digitonin, Dnase, and complete protease inhibitor cocktail in PBS) at 4°C for 40 min. The rat anti-TASK1 antibody was preincubated with anti-rat coated magnetic beads (Dynal) prior to DMP crosslinking. Alternatively, the rabbit anti-TASK1 antibody (Alomone) crosslinked with protein-A coated Dynal beads was used. Both the rat and the rabbit anti-TASK antibodies were equally potent for IP. Cell lysates were immunoprecipitated with antibody-crosslinked beads overnight. After immunoprecipitation, beads were washed 10 times beginning and ending with low-salt wash buffer (0.1% digitonin, 50 mM Tris-HCl [pH 8.0], and 150 mM KCl). During middle washes the salt content was increased to 500 mM KCl. TASK1 complexes were eluted and dissociated in 2% LDS sample buffer (Invitrogen) supplemented with 20 mM DTT at 37°C for 30 min, and analyzed by Western blot.

Electrophysiology

HEK-293 cells on a 6-well plate were transiently transfected with 0.4 μg of pCGI-TASK alone, or with both 0.4 μg of TASK and 0.4 μg of pAdC8I-Vpu. Whole-cell current recording was performed during day 1 to day 3 posttransfection, at room temperature (21–23°C). Due to the use of the bicistronic construct pCGI-TASK1, cells expressing TASK1 exhibited green fluorescence. Similarly, cells transfected with pAdC8I-Vpu expressed both Vpu and CD8. CD8-positive cells were identified by immunostaining with anti-CD8 Quantum Red conjugate antibody (clone UCHT-4, Sigma). Coexpression was determined by both green and red Fluorescence. Whole-cell currents of the transfected cells were recorded using an Axonpatch 200B patch-clamp amplifier and filtered at 5 kHz. The membrane potential was held at 0 mV, before applying a voltage step of 100 msec, starting from –100 to 80 mV with an increment of 20 mV every 2 s. The bath solution contained (in mM) NaCl, 140; KCl, 5; $Ca(OH)_2$, 2; $Mg(OH)_2$, 1; glucose, 10; and HEPES, 10 (pH 7.4). The internal pipette solution contained (in mM) NaCl, 5; KCl, 120; $MgCl_2$, 1; EGTA, 2; HEPES, 10; and Mg-ATP, 2.5 (pH 7.2). In differential blockade experiments, continuous perfusion began with (1) the bath solution, (2) the bath solution without K^+, (3) removal of K^+ and addition of 10 mM TEA^+, and last (4) removal of K^+ and addition of both 10 mM TEA^+ and 2 mM Ba^{2+}. Before each change of solution, the step protocol was applied for recording. Leak current was not subtracted throughout. The currents recorded at each voltage step were averaged among the mid-40 msec and divided by individual cell capacitance to express current densities (pA/pF). The Ba^{2+}-sensitive, TEA^+-insensitive TASK1 current component was determined by subtraction of the current-voltage relationships taken at the third and the fourth perfusion (Backx and Marbán, 1993). For measurements of pH-sensitive currents, continuous perfusion was switched between the bath solution (pH 7.5) and the same solution adjusted to pH 8.5. The pH-sensitive TASK-1 current was defined as the difference in currents at pH 7.5 and pH 8.5. Vpu-expressing, apoptotic cells were identified using annexin V markers and propidium iodide (Molecular Probes).

Virus Release Assay

HeLa cells plated in P25 flasks were transiently transfected at 50% confluency with 2–3 μg of a HIV-1 proviral construct (pNL4-3, pNL4-3/Udel), and with of one of the constructs: pCGI (0.7 μg), pCGI-HERG (0.7 μg), pCGI-TASK1 (0.7 μg), or pCGI-Ttm1 (0.18 μg) using LipofectAMINE 2000 (Invitrogen). Various quantities of pCGI-TASK1 and pCGI-Ttm1 (0.18 μg, 0.35 μg, 0.70 μg, and 1.4 μg) have been assessed for optimization in virus release assays. At specific time-points posttransfection, one-tenth of the culture medium was retrieved from each sample and filtered through a 0.22 mm syringe filter. The viral content in culture medium was determined by Coulter HIV-1 p24 Antigen Assay kit. Data from individual experiments were averaged and expressed in mean ± SEM. The K^+ channel effects on virus release were compared with respect to the control (cotransfection of pNL4-3 and pCGI). The comparisons were made using a Student's *t*-Test. $P < 0.05$ were deemed significant.

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